MOLECULAR CLONING OF *PSEUDOMONAS AERUGINOSA* PENICILLIN-BINDING PROTEIN 3 GENE AND PURIFICATION OF THE PROTEIN

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Nine penicillin-binding proteins (PBPs) were detected in *Pseudomonas aeruginosa* using ^{3}H-penicillin G. They were tentatively designated by molecular weight as follows: PBP1A - 90,000, PBP1B - 81,000, PBP2 - 66,000, PBP2a - 62,000, PBP3 - 58,000, PBP3a - 45,000, PBP4 - 42,000, PBP5 - 40,000 and PBP6 - 32,000. By affinity chromatography with 6-aminopenicillinic acid (6-APA) as a bio-ligand coupled to Affi-gel 10 (Bio-Rad) or N-hydroxysuccinimidyl-chloroformate-agarose (Sigma), *P. aeruginosa* PBPs were isolated, among which PBP5 was a major product. With a mono Q FPLC column in 0.6% Octyl-POE eluted with a NaCl gradient from 0 to 1 M, PBP1A, 1B, PBP2 and 2a were separated from PBP3, 3a, PBP4, PBP5 and PBP6. Further chromatography using FPLC is needed to purify PBP3 and each of the other PBPs.

A pool of degenerate primers was designed from the conserved functional motifs SXXK and KTG of *E. coli* and *Neisseria* high MW PBPs (1). These primers were used for a PCR amplification experiment using *P. aeruginosa* chromosomal DNA as the template. A PCR product of 600 bp in length showed 70% homology to both *E. coli* PBP3 and *Neisseria* PBP2. The homology of these two proteins to a second PCR product (600 bp) was 55%. The nucleotide sequences of these two PCR fragments are not identical. Probing southern of *P. aeruginosa* chromosomes confirmed the different origins of these two fragments. These data implied that both or either of these PCR products might be the *P. aeruginosa* PBP3 gene(s). If the former assumption turns out to be true, the PBP3 gene may be duplicated in *P. aeruginosa*. Cloning of *P. aeruginosa* PBP3 gene(s) with these two 600 bp PCR fragments as the probes is currently being carried out.

1. Spratt, B.G. (1983) Penicillin-binding proteins and the future of β-lactam antibiotics. J. Gen. Microbiol. 129: 1247-1260

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